

16 and rather than considering claims 17-42 presented in the previous preliminary amendment please add and consider the following new claims 17-42, which are identical to the previously submitted claims 17-42.

17. (New) A method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from a solid tissue, with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

- a. coating paramagnetic particles or beads with an antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell and not on a non-target-cell in the cell mixture;
- b. mixing the coated paramagnetic particles or beads with the cell suspension containing the target-cells;
- c. incubating the mixture under gentle rotation;
- d. examining the target-cells after incubation; and
- e. counting the target-cells after incubation.

18. (New) The method of claim 17, wherein the paramagnetic particle or bead is coated with a murine or a human antibody or fragment thereof.

19. (New) The method of claim 17, wherein incubating lasts for 5-10 minutes to 2 hours.

20. (New) The method of claim 19, wherein incubating lasts 30 minutes.

21. (New) The method of claim 17, wherein incubating is at a temperature between 0°C and 25°C.

22. (New) The method of claim 21, wherein incubating is at a temperature about 4°C.

7 23. (New) The method of claim 17, wherein when the target cell population is contained in blood or bone marrow aspirates, the method further comprises the step of:

pre-incubating the antibody-coated paramagnetic particle and the cell suspension with mild detergent.

u
43100
8 24. (New) The method of claim 23, wherein the preincubating comprises as detergent Tween 20TM at a concentration less than 0.1% and the preincubation lasts 30 minutes at 4°C.
(polyoxyethylene sorbitan mono laurate)

9 25. (New) The method of claim 17, wherein when the density of target-cells is low, or when the ratio of target cell/total cells in the cell mixture is low ($\leq 1\%$), the method further comprises the step of:

subjecting the incubated paramagnetic particle-antibody-cell mixture to a magnetic field.

10 26. (New) The method of claim 25, wherein the particle-target-cell complexes are stained.

11 27. (New) The method of claim 17, wherein the step of examining, the step of counting, or both steps comprise using a microscope or a cell or particle counting device.

12 28. (New) The method of claim 17, further comprising the steps of:
isolating the target-cells by exposing the complex of cells and paramagnetic particles to a magnetic field to magnetically aggregate the cells;
subjecting the magnetically aggregated cells to further biological, biochemical, and immunological examination.

13 29. (New) The method of claim 17, wherein the antibody or fragment thereof is directed against an antigen or a receptor in a cell with abnormal developmental patterns

¹³
¹⁴ 30. (New) The method of claim ¹³29, wherein the cell is a primary or a metastatic cancer cell.

¹⁶
³¹ 31. (New) The method of claim ¹17, wherein the antibody or fragment is of IgG isotype, a F(ab')₂ fragment, a F(ab) fragment, IgM, or a fragment of IgM.

¹⁷
³² 32. (New) The method of claim ¹17, wherein the cell suspension or population comprises mammalian tissue, a pleural effusion, a peritoneal effusion, a body fluid, or a solid tumor in a normal tissue or organ.

¹⁷
³³ 33. (New) The method of claim ¹⁷32, wherein the mammalian tissue comprises human bone marrow or human peripheral blood; the body fluid comprises urine, cerebrospinal fluid, semen, or lymph; or the normal tissue or organ comprises liver, lymph node, spleen, lung, pancreas, bone, central nervous system, prostate gland, skin, or mucous membranes.

³⁴ 34. (New) The method of claim 17, wherein the antibody or antibody fragment is directed against fibronectin receptor, β -integrin, vitronectin receptor, $\alpha\beta$ 3-integrin, P-selectin, GMP-140, CD44-variants, N-CAM, E-cadherin, Le^x, CEA, EGF receptor, c-erbB-2, HER2, transferin receptor, TNF-receptor, high molecular weight antigen (HMW 250,000), p95-100, TP-1 and TP-3 epitope, Mv 200kD, Mv 160kD, MOC-31 epitope, cluster 2 epithelial antigen, MUC-1 antigen, DF3-epitope, gp290kD, prostate high molecular antigen (Mv > 400kD), TAG 72, bladder carcinoma antigen (Cancer Res. 49, 6720, 1989), Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, β_2 -microglobulin, Apo-1 epitope, or pan-human cell antigen.

35. (New) The method of claim 17, wherein the antibody or antibody fragment is directed against a growth factor receptor and or an oncogene product expressed on the membrane of a malignant cell

36. (New) The method of claim 35, wherein the antibody or antibody fragment is directed against an insulin receptor, an insulin-like receptor, or FGF.

37. (New) The method of claim 29, wherein the antibody or antibody fragment is directed against an adhesion membrane molecule or an MDR proteins in the abnormal cell.

¹⁵
~~38.~~ (New) The method of claim ¹³~~29~~, wherein the antibody or antibody fragment is directed against breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

¹
cont. [39. (New) The method of claim 29, wherein the antibody or antibody fragment is directed against cells associated with a non-neoplastic disease.

40. (New) The method of claim 39, wherein the non-antineoplastic disease affects a cardiovascular cell, a neurological cell, a pulmonary cell, an autoimmune cell, a gastrointestinal cell, a genitourinary cell, or a reticuloendothelial cell.

¹⁹
~~41.~~ (New) A kit for performing the method of claim ¹~~17~~, the kit comprising:
a. a specific antibody or antibody fragment directed to an antigen on a target-cell, which antibody or fragment is effective for coating a paramagnetic particle or bead without removing its antigen-binding ability;
b. a paramagnetic particle or bead; and
c. another specific antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;

wherein said another antibody or antibody fragment is conjugated to biotin or to an enzyme; or wherein said another antibody or antibody fragment is bound to a non-paramagnetic particle with a specific color or with a bound enzyme.

D'
encl. ²⁰₄₂ (New) The kit of claim ¹⁹₄₁, wherein the enzyme is peroxidase or alkaline phosphatase.

It is believed that this application is now in condition for examination. Such action is respectfully requested.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402
612/332-5300

By

Mark T. Skoog
Mark T. Skoog
Reg. No. 40,178